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(21) International Application Number: PCT/AU92/00200 (22) International Filing Date: 1 May 1992 (01.05.92) (30) Priority data: PK 5970 6 May 1991 (06.05.91) AU (71)(72) Applicant and Inventor: GUERRINI, Vincent, Henry [AU/AU]; 173 Chatswood Road, Daisy Hill, QLD 4128 (AU). (81) Designated States: AT, AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH, CH (European patent), CI (OAPI patent), CM (OAPI patent), CS, DE, DE (European patent), DK, DK (European patent), ES, ES (European patent), FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), GN (OAPI patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LK, LU, LU (European patent), MC (European patent), MG, ML (OAPI patent), MN, MR (OAPI patent), MW, NL, NL (European patent), NO, PL, RO, RU, SD, SE, SE (European patent), SN (OAPI patent), TD (OAPI patent), TG (OAPI patent), US.		Published <i>With international search report.</i> <div style="text-align: right; font-family: cursive; font-size: 1.2em;"> 08/992,515 2000/01 </div>
(54) Title: IMPROVEMENTS OF A PESTICIDAL/INSECT REPELLENT COMPRISING SYNTHESIS OF A NEW PESTICIDAL INSECT REPELLENT (57) Abstract <p>There is provided a novel method to improve the pesticidal and repellent properties of neem extracts or azadirachtin containing compositions comprising the synthesis of a new pesticide repellent. Neem extracts containing azadirachtins or compositions containing neem extracts or neem extracts with azadirachtins, dialkyltoluamides and volatile oils are heated between 30 and 200 degrees centigrade from 1 second to 1000 hours to improve the pesticidal and repellent properties of the compositions or synthesize new pesticides or insect repellents.</p>		

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IMPROVEMENTS OF A PESTICIDAL/INSECT REPELLENT COMPRISING SYNTHESIS OF A NEW PESTICIDAL INSECT REPELLENT.

The present invention relates to improvements of pesticidal compositions comprising methods to synthesize or produce a new pesticidal/insect repelling substance.

In Australian Patents 608281, 608564, 607922, and Patent application 26320/88, the present applicant describes the use of azadirachtin rich extracts of neem seeds and other meliaceous plants to inhibit pests such as blowfly larvae, lice and fleas.

Azadirachtin rich extracts from neem seeds have been used in Indian folk medicine for thousands of years and this, together with recent laboratory tests has shown neem seed extracts or meliaceous extracts with azadirachtin to be of very low toxicity to vertebrates.

It is an object of this invention to provide a new hereto unknown pesticidal/insect repelling substance and to provide a method to synthesize or produce the new insect or pest inhibiting substance. It is a further object of this invention to provide a method to improve the insecticidal/insect repelling properties of the pesticidal composition described in Patent PCT/AU90/00586.

The present invention thus provides a new pesticidal/insect repellent substance and an improved pesticidal/insect repellent mixture by mixing 1 to 35% by volume of oil of citronella, from 2 to 30g per litre azadirachtin, from 30 to 200g per litre neem seed extract or extract of meliaceous plants containing 5% azadirachtin, from 30 to 200g per litre diethyl-

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toluamide (DEET), from 6 to 46g per litre di-n-propyl isochinchomerate. from 1.5 to 10.5g per litre N-Octyl bicycloheptene dicarboximide, from 0.17 to 1.16 g per litre Tri-closan and alcohol and lemon grass oil the remainder, and heating the mixture to between 30 and 200 oC from 1 second to 1000 hours.

The invention also relates to a method of killing or repelling pests comprising applying the mixture and new molecule to the pests or their surroundings. The substances may be applied together or sequentially.

The invention also relates to a method of synthesizing Azadirachtins, nimbidens, protoliminoids, liminoids, or tetratriterpenoids, pentanortriterpernoids, including meliantrol, nimbocinone, azadirachtol and azadirachnol or changing the structure of the compounds shown on Drawing 1, page 1/3, present in extracts of meliaceous trees including neem tree extracts (*Azadirachta Indica* A.Juss.) or which have been synthesized artificially.

The invention also relates to a method or a process to increase the concentrations of the aforementioned substances present in meliaceous tree extracts including neem tree and seed extracts namely the tetratriterpenoids, pentanortriterpenoids, hexatriterpenoids, azadirachtins, salanninns, azadiradiones, nimidens and vilanisin and derivatives. These substances are illustrated on Drawing 1. page 1/3.

The invention also relates to a method or process to unbind or release the aforementioned substances illustrated on

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Drawing 1, page 1/3 from proteins, fats, oils or any other substances to which the substances illustrated on Drawing 1, page 1/3 bind in the extracts of meliaceous trees including neem seeds and extracts.

The invention also relates to a process or method to improve the pesticidal and insect repellent properties of the substances illustrated on Drawing 1, page 1/3.

The invention also relates to a method to change the structure of the substances shown on Drawing 1, page 1/3, wherein the extracts of meliaceous trees including neem seed extracts are heated in absence or presence of air to between 30oC and 200oC for periods ranging between 1 second and 1000 hours.

The invention relates to a process or method to increase the concentrations, to unbind and improve the pesticidal and insect inhibiting properties of the substances shown on Drawing 1 page 1/3 wherein the extracts of meliaceous trees including neem tree and seeds extracts are heated in the presence or absence of air to between 30oC and 200oC for periods ranging between 1 second and 1000 hours.

Further embodiments of the invention shall become apparent from reference to the examples.

Example A

One litre of a liquid pesticidal/insect repelling composition may be made by mixing the following:-

Oil of citronella	10	to	350ml
Meliaceous extracts containing 5% azadirachtin	40	to	600ml

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or pure azadirachtin	2	to	30g
Triclosan	0.17	to	1.16g
N,N,Diethyl-M-Toluamide	30	to	200g
Di-N-propyl Isochinchomerate	6	to	46g
Alcohol (e.g. Ethanol)	Remainder		
Lemon Grass oil	Traces		

Comparitive Example 1a

Four samples of the mixture were kept at 40C for 14 days (Samples 1) and four samples were kept at 540C for 14 days (Samples 2).

0, 5, 20, 60, and 100 drops respectively, of Samples 1 or Samples 2 were blended with 10 grams of food containing 50 first stage larvae of the insect *Lucilia cuprina*. Each drop contained 5 microlitres (ul) of liquid.

Larval length or growth and larval survival in food blended with Samples 1 or Samples 2 are set out in Table 1a on the next page of this description.

Table 1a

Drops (number)	0	5	20	60	100
Dose (ul)	0	25	100	300	500

Samples 1\$
Mixture stored at
40C for 14 days

Larvae Length(mm)	8.0+0.3	6.8+0.5	3.3+0.3	1.2+0.8	0
Survival(%)	96	86	43	12	0

Samples 2\$
Mixture stored at
540C for 14 days

Larvae Length(mm)	8.0+0.3	3.2+0.2*	0*	0*	0*
Survival(%)	96	11	0	0	0

\$ = All tests duplicated (ie: Four samples for each concentration n=4)

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* = $P < 0.05$ larval length and survival was significantly less in samples 2 compared to samples 1 (Two-way analysis of variance).

From a comparison of results in Table 1a it is apparent that heating at 54°C for 14 days increased the pesticidal/insect inhibiting properties of the mixture. In fact, heating increased efficiency 53 to 100% over the range of concentrations tested.

Table 1b

Concentration of Active constituents including azadirachtins, nimbins, triterpernoids, pentatriterpernoids, salannins vilasinins, liminoids in neem seed extracts heated between 30°C and 70°C for 1 second to 1000 hours.

	0 seconds	1000 hours
Concentration (%)	1	5

A comparison of results in the table 1b shows that the concentration of the actives in neem seeds extracts increased five fold when exposed to heating.

Comparative example 1b

Drawing 2 on page 2/3 (drawings) shows the tracings representing the concentration of azadirachtin and other substances in meliaceous extracts in samples 1 kept at 40°C for 14 days.

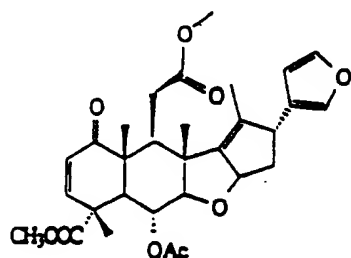
Drawing 3 on page 3/3 (drawings) shows the tracings representing the concentration of azadirachtin and other substances in meliaceous extracts in this case neem seed extract in Samples 2 kept at 54°C for 14 days

From a comparison of Drawing 2 and Drawing 3 it is apparent that heating the pesticidal/insect repellent mixture created a new unknown substance.

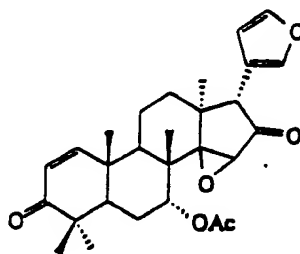
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DRAWING 1

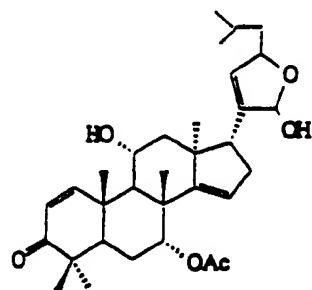
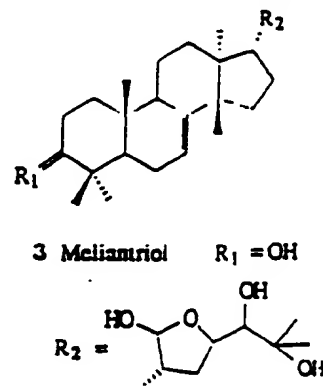
Structural formulae of triterpenoids found in extracts of the neem tree *Azadirachta Indica*.



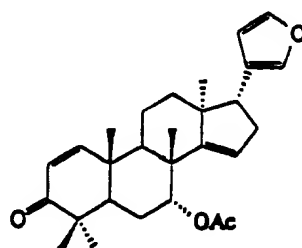
1 Nimbin



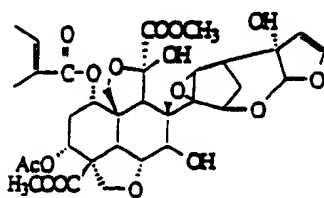
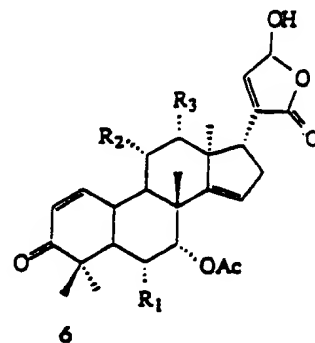
2 Nimbinin



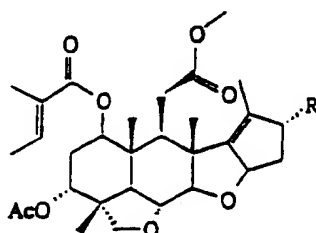
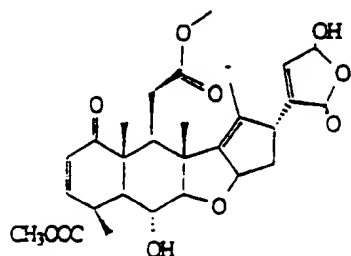
4 Azadirachtol



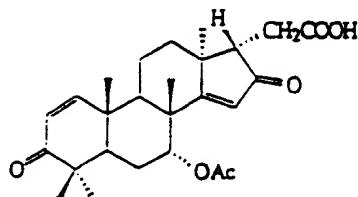
5 Azadirone



7 Azadirachtin

8a Salannin $R = \text{furan ring}$ 8b Salannolide $R = \text{furan ring with OH}$ 

9 Desacetylnimbinolide

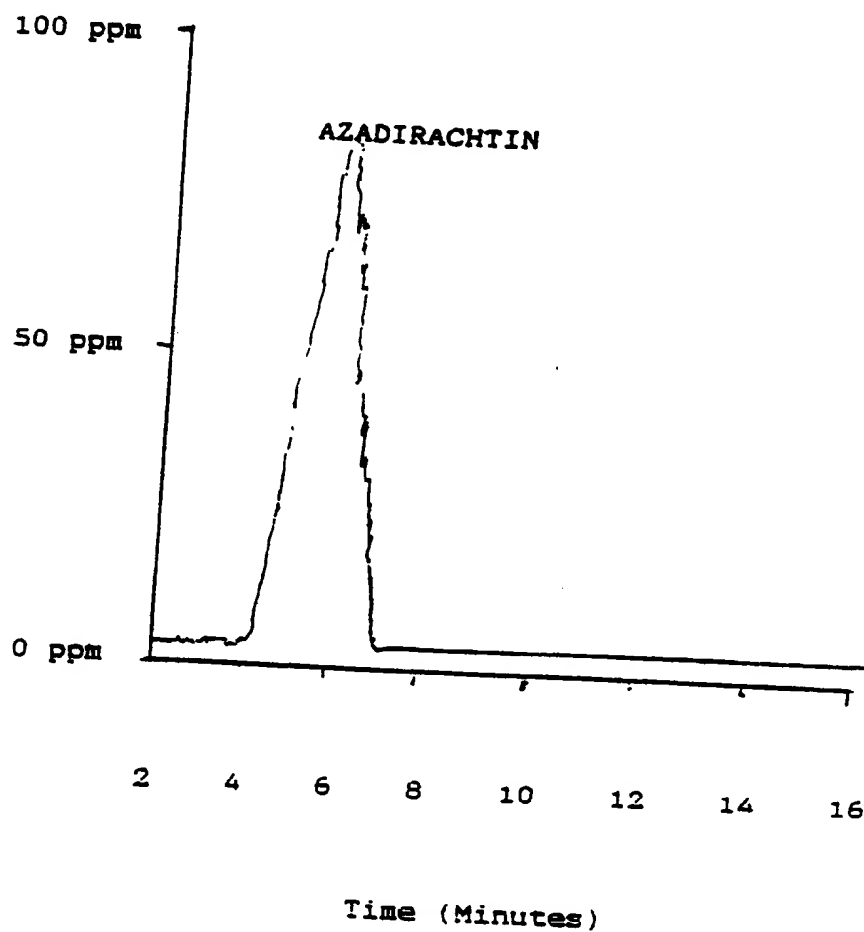


10 Nimolicinoic acid

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DRAWING 2

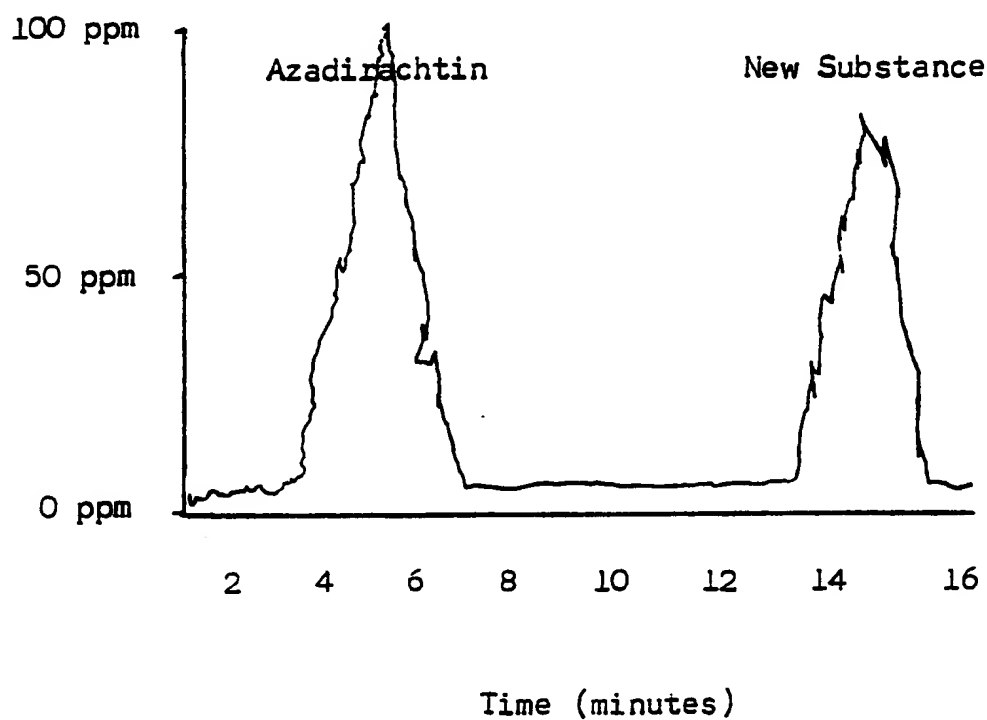
Tracing representing the concentration of
azadirachtin and other substances in meliaceous extracts
(neem seed extracts) kept at 40C for 14 days.



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DRAWING 3

Tracing representing the concentration of Azadirachtin and other substances in meliaceous extracts (neem seed-extracts) kept at 54 °C for 14 days.



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INTERNATIONAL SEARCH REPORT

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶		
According to International Patent classification (IPC) or to both National Classification and IPC Int. Cl. ⁸ C07D 407/08, 493/18, 493/06, C07J 71/00, 17/00, 19/00		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
DERWENT WPAT, CHEMICAL ABSTRACT CAS82 KEYWORDS: "MELICACEOUS, neem, Azadiracht: Melial indica, Margasa, Indiam lilac, Nimbin, Melliantrol".		
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched ⁸		
AU: IPC C07D 407/08, 493/18, 493/06, C07J 71/00, 17/00, 19/00		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹		
Category ⁹	Citation of Document, ¹¹ with indication, where appropriate of the relevant passages ¹²	Relevant to Claim No ¹³
X	AU,B, 23445/88 (609526) (ROHM and HAAS) 06 April 1989 (06.04.89)	1,4
X	EP, A, 308044 (NATIVE PLANTS INCORPORATED) 22 March 1989 (22.03.89)	1,4
X	US, A, 4902713 (Rembold et al) 20 February 1990 (20.02.90)	1,4
X	Phytochemistry, Vol 29, No. 12, pp3963-3965, 1990. Gaikwad et al; "NIMBOCINOL and 17- EPINIMBOCINOL FROM THE NIMBIDIN FRACTION OF NEEM OIL"	1,4
(continued)		
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>¹⁰ Special categories of cited documents :</p> <p>"A" Document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" Later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"Z" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search 31 July 1992	Date of Mailing of this International Search Report 17 Aug 1992 (17.08.92)	
International Searching Authority AUSTRALIAN PATENT OFFICE	Signature of Authorized Officer T. SUMMERS	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category*	Citation of Document, ¹¹ with indication, where appropriate of the relevant passages ¹²	Relevant to Claim No ¹³
X	TETRAHYDRON Vol 43, No. 12, pp 2805-2815, 1987. Bilton et al; "AN X-RAY CRYSTALLOGRAPHIC, MASS SPECTROSCOPIC, AND NMR STUDY OF THE LIMINOID INSECT ANTIFEEDANT AZADIRACHTIN AND RELATED DERIVATIVES.	1,4
X	TETRAHYDRON Vol 43, No. 12, pp 2817 to 2830, 1987. Kraus et al; "STRUCTURE DETERMINATION BY NMR OF AZADIRACHTA INDICA A. JUSS (MELIACEAE)"	1,4
X	Journal of the Chemical Society, PERKIN TRANS, Vol 1, pp 1429-32, 1987. Siddiqui et al; "STUDIES IN THE CHEMICAL CONSTITUENTS OF AZADIRACHTA INDICA A. JUSS (MELIACEAE). PART 10, ISOLATION AND STRUCTURE ELUCIDATION OF ISONIMOLICINOLIDE, THE FIRST 17-ACETOXY TETRANORTER-PENOID AND NIMOLICINOIL ACID, THE FIRST HEXANORTRITERPENOID WITH AN APOEUPHANE (APOTIRUCALLANE) SKELETON".	1,4

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

X	Phytochemistry, Vol 27, No 12 pp3903-3907, 1988. SIDDIQUI et al; "PHENOLIC TRICYCLIC DITERPENOIDS FROM THE BARK OF AZADIRACHTA INDICA"	1,4
X	Journal of Natural Products Vol 51, No. 1 pp30-43, 1988. SIDDIQUI et al; "TETRACYCLIC TRITERPENOIDS AND THEIR DERIVATIVES FROM AZADIRACHTA INDICA".	1,4
X	HETEROCYCLES, Vol 29, No. 1, 1989 pp 87-96, SIDDIQUI et al; "TETRANORTRITERPENOIDS FROM AZADIRACHTA INDICA A. JUSS (MELIACEAE)".	1,4
X	HETEROCYCLES, Vol 28, No. 1, 1989 Inada et al; "PHYTOCHEMICAL STUDIES ON MELICACEOUS PLANTS. STRUCTURE OF A NEW APOTIRCUALLANE - TYPE TRITERPENE, 21-O-METHYL TOOSENDANPENTOL FROM FRUITS OF MELIA-TOOSENDAN SIEB".	1,4

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

- ☐ Claim numbers ..., because they relate to subject matter not required to be searched by this Authority, namely:
- ☒ Claim numbers 8..., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Claim 8 is so unclear that this International Searching Authority considers that no meaningful search can be carried out on this claim.
- ☐ Claim numbers ..., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4a

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ²

This International Searching Authority found multiple inventions in this international application as follows:

Claims 1-3 define three different inventions, and claim 4 in so far as it does not relate to claim 1 defines a fourth invention.

- ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
- ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
- ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
1 and 4 (in part).
- ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.